CELL BEHAVIOR IN TISSUE CULTURES.

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This paper deals with certain free wandering cells observed in tissue cultures from the minnow, *Fundulus heteroclitus* and *Fundulus majalis*. The types of cells which became free and isolated from the spreading tissue growths were, chromatophores, amœboid mesenchyme cells and, most abundant of all, certain cells having curious fan shaped projections. These cells proved to be identical with those studied in their association in tissues in cultures by Dr. Dederer ('21). She identified these as mesenchyme cells which in cultures were the means of attachment of the outgrowing sheets of ectodermal cells to the surface of the coverslip. In the work here presented isolated cells were sought as the best objects for the study of cell behavior. The mode of locomotion and the tactile reactions were more especially studied and for the latter work the Barber microdissection apparatus was utilized. The tissues were cultivated in the sea water medium (M. R. Lewis, '16) using, however, in many cases more dilute solutions. Observations were usually made within two days after planting. The work was done at the Marine Biological Laboratory at Woods Hole, Massachusetts during the summers of 1921 and 1922. I wish to acknowledge my indebtedness to Mrs. D. B. Young for the drawings from the stained preparations and to Mr. S. C. Williams for aid in certain of the observations on the rate of motion of cells.

The Fan Cells—Among cells of this type an abundant form was that for which I came to use the descriptive term "Canoe cells." When first observed these seemed to be elongate spindle-shaped cells such as indicated by many outlines in Fig. 4. I supposed that there were delicate psuedopedia at either end but upon plotting the direction of the motion of these cells I was surprised to find that they were moving steadily at right angles to the long axis. More careful observations upon living and upon fixed and stained preparations showed the presence of a delicate

film along what proved to be the anterior side of the cell. This film or fan rounded about the ends of the elongate cell, thus giving the canoe-like form (Fig. 1). By use of the microdissection

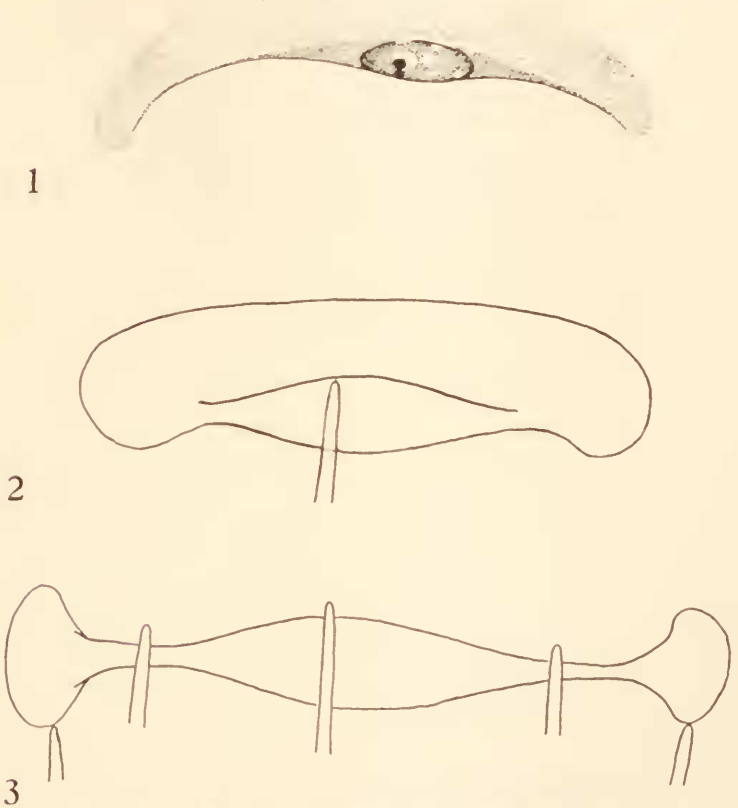

FIG. 1. Typical "Canoe" cell. Drawn from stained preparation.

FIG. 2 and 3. Diagrams of "Canoe" cell (2) and double fanned or bipolar cell (3) showing microdissection needle above body of cell and beneath the cover glass. The fans are firmly attached and the needle can not be pushed between them and the glass.

apparatus the relation of the cell to the cover glass was determined more accurately. It was possible to slide a needle between the more visible spindle shaped part of the cell and the cover glass. The, fan, however, was firmly attached (Fig. 2). The fan was clearly of ectoplasm in the gel state while there was more fluid protoplasm within the body of the cell.

I believe these fans to be the motor organs of the cell. There

seemed always to exist a definite relation between the position of the fan and the direction of motion. The fans were the only portion of the cells in contact with the solid support and there seemed to exist no mechanism for locomotion in a fluid medium without support. Other types of fan cells, as described below, illustrate these points even more clearly than the "Canoe" cells. The rate of motion of the "Canoe" cells was studied by plotting their course with a camera lucida. Fig. 4 shows the history of such a cell. In most cases the outline of the fan could not be observed with the camera in position and an outline of only the body was drawn. However, in positions 1, 12, 21, 28, 30, 32 and 34 the probable form of the fan is indicated by dotted lines. These outlines were based on observations with the prism of the camera removed. At position 8 the cell became attached by a psuedopodium-like projection on the right which may have terminated in a fan. A similar process occurred at positions 24 to 35 during which period a small fan could clearly be observed at the right. At position 27 the cell under observation collided with another cell. The two cells became attached and the newcomer formed an irregular projection at the upper right of the original cell (positions 27 to 36).

The average rate of motion of freely moving cells excluding such cells as proved to be slowing down prior to the death of the cell, was 6.3 microns per minute. The cell shown in Fig. 4 moved at a rate of 5.3 microns from positions 1 to 25. An attempt was made to study the effects of changes of temperature upon the movement of these cells. For this purpose the cultures were studied under the microscope in a warmed box at temperatures varying from 21° centigrade to 42°. Above 40° the cells withdrew their fans and became rounded. Observations were made at constant temperatures and also during an increase of temperature. It soon became apparent that variations in the conditions of individual cells would preclude the possibility of constructing a temperature curve for the rate of locomotion. Cells becoming free from the main growth of tissue moved for a variable period and then became rounded and this condition probably preceeded the death of the cell. A slowing of the rate of motion was apparent before the contraction took place. Also the varying form of the cell and the probable occasional attachment by small sub-

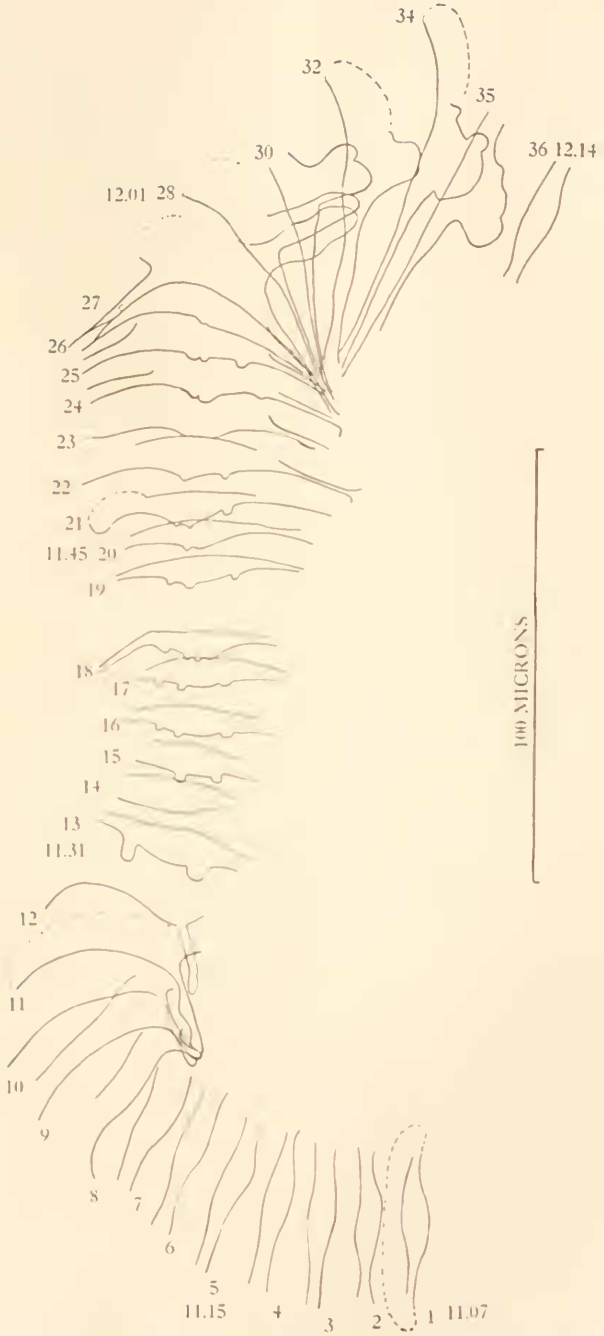

FIG. 4. The history of a "Canoe" cell for one hour and seven minutes at room temperature. The solid lines outline the cell body in so far as it was visible when projected by the camera lucida. The dotted lines show boundary of fans based on observations with the prism of the camera raised.

sidiary fans, as in Fig. 4, which are invisible when making camera drawings, doubtless influence the rate of motion. Nevertheless the results seem to indicate that an increase of temperature causes an increase in the velocity of the movement. The average of all (15) observations at room temperature showed a rate of locomotion of 6.3 microns per minute. The average of all (10) observations at higher temperatures (in most cases varying) was 7.3 microns per minute. The records of greatest speed were 11.5 microns and 10 microns per minute and were attained by cells at the higher temperatures. In the latter case the cell was followed for 39 minutes. The details of these observations are recorded in the tables in the appendix to this paper.

A second form assumed by these cells was that exhibiting two fans (Figs. 5, 6, 7). These fans were usually at opposite poles and under their influence the cell became greatly attenuated. In such cases a micro-dissection needle could be passed between the coverslip and the body of the cell (Fig. 3), but the fans were found to be firmly attached. The cell was thus freely suspended like a hammock between two supports—the fans forming means of attachment and also of extension. Two typical double fanned or bipolar cells are shown in Fig. 5 and 7 which were drawn from stained preparations. In Fig. 6 are shown two cells attached with one fan pulling in a direction not directly opposed to the other.

Another extraordinary mode of motion was observed in which the contractility of the cell functioned as well as the gliding motion of the fan. The history of such a cell is shown in Fig. 9. The account begins at 2.44 P.M. with the cell at position 1 and with a fan at either end. Suddenly a release of the fan occurs and the cell contracts and is thrown into position 2. It again elongates, fans are found at either end—positions 3, 4, 5 and at 3.01 a second contraction occurs, throwing the cell into position 6. The process is repeated four times—positions 6–9, 9–12, 12–13, and 13–18. In each case the cell is elongated by the pulling of the opposed fans. This unusual mode of motion was observed in a culture from a 17-day embryo. This marked contractility of a mesenchyme cell is almost suggestive of the behavior of muscle cells as described by M. R. Lewis ('20) except that these cells

FIGS. 5, 6, 7, 8. Typical fan cells drawn from stained preparations. Figs. 5 and 7 are bipolar or double fanned cells. The limits of the fans may have extended further than indicated. Fig. 6. Fan cells—possibly shortly after cell division. Fig. 8. Two cells apparently fused forming syncytium. Magnification about 1800 times.

contract completely to a spherical form and then gradually expand.

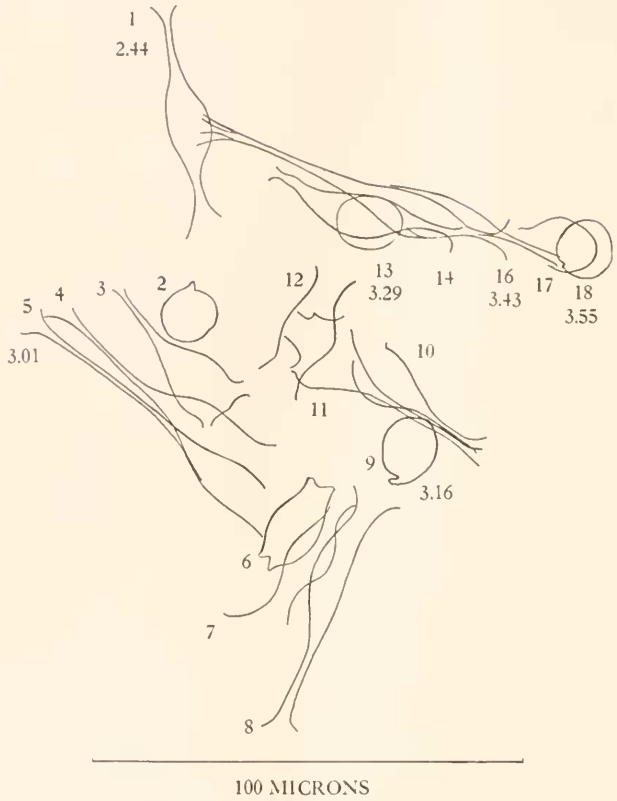

FIG. 9. The history of a bipolar fan cell for one hour and eleven minutes at room temperature of 23°. Movement is by method of alternate expansion and contraction. For details see text.

The cells having fans were found only in cultures showing epithelial growths such as described by M. R. Lewis ('16) and Dederer ('21). I have planted cultures from embryos of various stages but have been unable to obtain this type of growth from those of less than six days of age when developing at the temperature of the running sea water in the laboratory (19° to 22°). The most favorable period for planting and obtaining such growths is shortly before hatching (about 17 days, 19°–22°). Sections of embryos of these later stages reveal a conspicious layer of cells beneath the surface epithelium which are almost completely

wanting in the earlier stages. Dr. Dederer's observations have shown the close relation between epithelial cells and the fan cells and it is then not improbable that this layer contains the fan cells. Moreover she has shown these fan cells to be necessary for the spreading of the epithelial growth by attaching it to the cover glass. Therefore the absence of the epithelium in cultures from the younger embryos may be due to the absence of this sub-epithelial layer with its included fan cells.

In a few cultures other unpigmented cells of presumably mesenchymal origin and of exceedingly irregular form were noted. Their mode of motion seemed typically amœboid.

Tactile Reactions.—The tactile reactions of various types of cells were studied. For this purpose the cells were touched with a delicate glass needle moved by a Barber micro-dissection apparatus. If the body of a double fanned cell such as that shown in Fig. 3 is sharply stimulated the fans are released and the whole cell contracts, becoming spherical. A fan may be pried loose and a similar reaction ensues. The contraction seems in part due to an elastic tension of the cell. I have not found it possible to stimulate a cell by touching or even partially mutilating a small portion of the fan. The ectoplasm does not seem to conduct a stimulus. In some cases a delicate stimulus near the boundary of a fan and the cell stalk of a greatly elongated cell will cause complete contraction. The material of the fan seems to flow together, making a ball of protoplasm which is carried along with the stalk to the center of the cell.

I have tried a few experiments to determine the chemotactic response of such tissue cells. A cloud of methylene blue injected by a micro-pipette will cause the contraction of these isolated cells. I have not succeeded in obtaining any more definite response such as a change in the direction of motion.

Previous writers, Bancroft ('12), Stockard ('15) and Newmann ('18) have described two types of chromatophores in the *Fundulus* embryo. These are black chromatophores or melanophres and the brown (or red) chromatophores. Bancroft ('12) has described a third type which also appeared in these tissue cultures. These were yellow and smaller than the other types and showed few or no pseudopodia. As a group, the chromatophores are but slightly responsive to tactile stimulation. If a needle is pushed against

a pseudopod with sufficient force to slightly indent the ectoplasm, the pseudopod may slowly withdraw. A brownian movement of pigment granules is frequently initiated. Of these cells the yellow chromatophores are most responsive, the red are less responsive and the melanophores are almost completely inert to tactile stimulation.

All cells studied seem to show less variety of adaptive response than the amœba. Thus the only reaction that I have observed is contraction either of the whole or a portion of a cell.

I have previously referred (Goodrich, '22) to the motion of the fan cells as non-amœboid. The characteristic streaming of protoplasm which we associate with the amœba is certainly not present. The phenomenon seems more akin to the movement of diatoms. It is, however, not impossible that this gliding motion may be a factor in the locomotion of many unicellular organisms. Schaefer ('20) in his discussion of amœboid movement has called attention to the importance of a surface film of streaming protoplasm external to the ectoplasm and wholly distinct from the familiar streaming of the endoplasm. This film can be observed only indirectly as it carries particles that become entangled in it. Schaefer states (page 106) that "the surface film in amœbas is powerful enough to enable them to move by it." In this case it causes a backward motion. It is also probable (see Schaefer, '20, for discussion) that such a surface film is important in the movement of diatoms, *Oscillitoria* and even of Gregarines. No adequate explanation has been offered for the motion of this surface film. I have not been able to detect the presence of such a moving film in the fans of the cells studied in this paper although cells have been observed in media containing a suspension of carbon granules. Yet the delicacy of the fan is such as to make the test seem inadequate and it is not impossible that such a mechanism may exist. If so we may have some clue to the motion of many cells in development and in regeneration. Moreover this mode of motion seems allied to the power of adhesion of cells. The cells here described are attached to the cover glass by means of the fans. Dr. W. H. Lewis ('22) has raised the question as to why tissue cells adhere in an organism. The mechanics of the gliding motion seems to involve this power of adhesion and thus the two problems may be related.

SUMMARY.

1. Certain isolated cells from tissue cultures of *Fundulus* embryos have been described.

2. These cells posess fan-shaped films which are adherent to the cover glass.

3. These films are the motor organs of the cells by means or which they glide on the under surface of the cover glass.

4. The tactile reactions of these cells and of the chromatophores are described.

5. The relation of this type of cell movement to amœboid movement is discussed.

BIBLIOGRAPHY.

Bancroft, F. W.

'12 Heredity of Pigmentation in *Fundulus* Hybrids. Jour. Exp. Zoöl., Vol. 12, No. 2.

Dederer, Pauline H.

'21 Behavior of Cells in Tissue Cultures of *Fundulus heteroclitus* with Special Reference to the Ectoderm. BIOL. BULL. Vol. 41, No. 4.

Goodrich, H. B.

'22 Cell Behavior in Tissue Cultures. Abstract from proceedings of Am. Soc. of Zoologists. Anat. Rec., Vol. 23, No. 1, p. 100.

Lewis, M. R.

'16 Sea Water as a Medium for Tissue Cultures. Anat. Rec., Vol. 10, No. 4.

'20 Muscular Contraction in Tissue Cultures. Carnegie Institution of Washington. Contributions to Embryology, No. 35. Publication No. 272.

Lewis, W. H.

'22 The Adhesive Quality of Cells. Anat. Rec., Vol. 23, No. 7.

Newmann, H. H.

'18 Hybrids between *Fundulus* and Mackerel. Jour. Exp. Zool., Vol. 26, 1918, No. 3.

Schaeffer, Asa A.

'20 Amœboid Movement. Princeton University Press.

Stockard, Charles R.

'15 A Study of Wandering Mesenchymal Cells on the Living Yolk Sac and their Developmental Products; Chromatophores, Vascular Endothelium and Blood Cells. Am. Jour. Anat., Vol. 18, No. 3.

APPENDIX.

TABLE I.

RECORD OF OBSERVATIONS ON RATE OF MOVEMENT OF CELLS AT ROOM TEMPERATURE.

Reference Number.	Average Rate in Microns per Minute.	Temperature in Degrees Centigrade.	Duration of Observations in Minutes
1	3.4	24.5	41
2	3.3	24.5	42
3	7.6	24	16
4	4.	24.	33
5	4.1	24.	19
6	5.9	24.	11
7	8.1	22.5	25
8	8.9	22.	15
9	8.2	22.	11.5
10	7.	22.	8.5
11	6.1	22.	9.
12	8.	21.5	20
13	7.7	21.5	13
14	8.	21.5	7.5
15	5.3	22.	48.

TABLE II.

RECORD OF OBSERVATIONS OF RATE OF MOTION OF CELLS AT HIGHER TEMPERATURES.

The temperature was in most cases gradually increased as indicated.

Reference Number.	Average Rate in Microns per Minute.	Temperature in Degrees Centigrade.	Duration of Observations in Minutes.
16	8.3	29.5–37	25
17	11.5	27 –33.8	20
18	8.3	33.8–36.5	25
19	3.4	27–35	44
20	9.1	35	7
21	5.	27–30	12
22	5.8	28–31	8
23	6.4	27–31	13
24	5.1	27–29	10
25	10.	33–35	39